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Amplification of RNA by an RNA Polymerase Ribozyme

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Introduction: In all extant life, genetic information is stored in nucleic acids that are replicated by polymerase proteins. In the hypothesized RNA world, prior to the evolution of genetically-encoded proteins, ancestral organisms contained RNA genes that were replicated by an RNA polymerase ribozyme [1,2]. In an effort toward reconstructing RNA-based life in the laboratory, in vitro evolution was used to improve dramatically the activity and generality of an RNA polymerase ribozyme by selecting variants that can synthesize a variety of complex structured RNAs, including aptamers, ribozymes, and, in low yield, even tRNA. The polymerase can also transcribe, with more modest activity, template RNA into nucleic acid analogs, including threose nucleic acid (TNA). Furthermore, the polymerase can replicate nucleic acids, amplifying short RNA templates by more than 10,000-fold in an RNA-catalyzed form of the polymerase chain reaction (riboPCR, fig. 1B). Thus the two prerequisites of Darwinian life — the replication of genetic information and its conversion into functional molecules — can now be accomplished with RNA in the complete absence of proteins. Currently, polymerases are undergoing in vitro evolution to synthesize complete functional ribozymes within minutes, with the aim of achieving fully autonomous RNA replication of the polymerase itself and other ribozymes of similar complexity. Such a general RNA replicase could, under appropriate conditions, achieve self-sustained Darwinian evolution and would arguably constitute a synthetic form of RNA life.

References: [1] Gilbert W (1986) *Nature* 319(6055):618. [2] Joyce GF (2002) *Nature* 418(6894):214–221. [3] Horning DP and Joyce GF (2016) *Proceedings of the National Academy of Sciences USA*, 113(35):9786-9791.

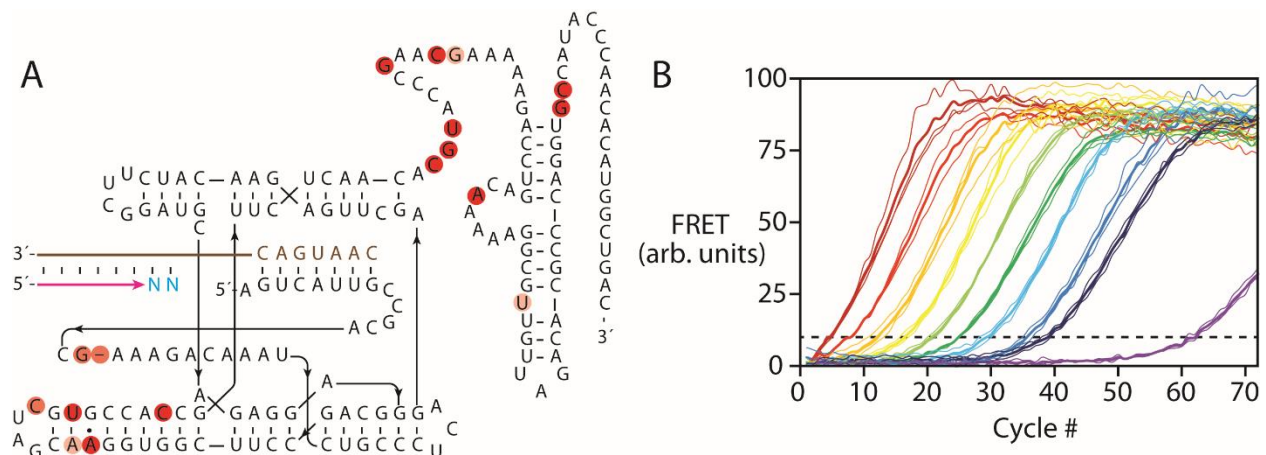


Figure 1 – (A) Sequence and secondary structure of a polymerase ribozyme isolated after 24 rounds of in vitro evolution. Red circles indicate mutations relative to the parental sequence, with color intensity corresponding to the frequency of each mutation in the round 24 population. (B) Real-time riboPCR of a 20-nt template, tracked by FRET between labeled primers in the dsRNA product. Starting template concentration spanned four orders of magnitude, from 10 nM (dark red) to 1 pM (indigo), while primers were at 200 nM.